Journal of Shellfish Research, Vol. 18, No. 2, 651–656, 1999.

FEEDING ENRICHED *ARTEMIA* BIOMASS TO *PENAEUS VANNAMEI* BROODSTOCK: ITS EFFECT ON REPRODUCTIVE PERFORMANCE AND LARVAL QUALITY

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ABSTRACT Two experiments were conducted co-feeding *Penaeus vannamei* broodstock with frozen *Artemia* biomass. In the first experiment, animals were fed natural diets supplemented with squid (treatment SQ), *Artemia* (A), or enriched *Artemia* (EA). In the second experiment, animals received a supplement of *Artemia* enriched with different products; rich in polyunsaturated fatty acids (PUFA) and cholesterol (treatment L), rich in vitamin c, vitamin e, and astaxanthin (treatment V), or a complete enrichment (treatment LV). In experiment 1, treatment SQ gave poor results for most parameters. Supplementation with *Artemia* resulted in higher survival, higher maturation frequency, a higher incidence of repeated spawns, and an improved larval quality. The best results were obtained in the treatment that received enriched *Artemia*. In experiment 2, the highest reproductive performance was obtained through enrichment of *Artemia* with both lipids and vitamins (LV). By reducing the concentration of PUFA and cholesterol in the enrichment product, a decline in egg fertilization, a lower incidence of repeated spawns, and a lower egg production per female was observed. High vitamin levels played a positive role only when provided in combination with high levels of PUFA and cholesterol. If not, symptoms of oversaturation occurred.

KEY WORDS: *Artemia*, reproduction, *Penaeus*, shrimp broodstock, nutrition

INTRODUCTION

In Ecuador—the world's second largest shrimp producer in 1997—stocking of growout ponds depends largely on wild *Penaeus vannamei* postlarvae (PL) and to a lesser extend on PL grown in hatcheries. Until recently, wild PL were preferred over hatchery PL by all the farm managers. Today, thanks to the progress made in reproduction and larviculture techniques, many managers consider both PL types of equal quality, and have begun to focus on closing the shrimp life cycle. In June 1998, 30 hatcheries had their proper maturation facilities and four more were under construction in Ecuador. This evolution spawned an urgent and increased need for applied research and technical assistance. Cost and availability of maturation diets are among the major problems in shrimp maturation. All maturation units base the nutrition of their reproducers on a mixture of fresh frozen natural diets (squid, mussel, oyster) locally available, with supplements of bloodworm imported from Maine, USA or Panama and relatively small portions of commercial dry diets. Traditionally, bloodworm has been the key to success in *P. vannamei* maturation; however, it is the most expensive component, and quality product is not available yearlong. A search for alternatives pointed toward *Artemia* biomass. Naessens et al. (1997) demonstrated that the replacement of bloodworm with adult *Artemia* does not negatively affect the reproductive performance of *P. vannamei*. In 1997, fresh-frozen enriched biomass of *Artemia* from the USA and occasionally from Peru was available on the Ecuadorian market and substituted completely or partially the bloodworm supplement in many of the hatcheries.

The effect of bloodworm on shrimp maturation has been attributed to its polyunsaturated fatty acid (PUFA) profile (Middleditch et al. 1980, Lytle et al. 1990). Although Browdy et al. (1989) and Naessens et al. (1997) demonstrated the importance of co-feeding *Artemia* to *P. semisulcatus* and *P. vannamei* broodstock, respectively, it remains unclear what constituents are responsible for triggering maturation. Therefore, more detailed research on *Artemia* enrichment components is needed. The present study consisted of two experiments. The first experiment was run from July until September 1996 and sought to identify which reproductive parameters are affected by *Artemia* biomass and enriched *Artemia* biomass, respectively. The second experiment was run from March until May 1997 and sought to identify the relative importance of certain enrichment nutrients.

MATERIALS AND METHODS

Experiment 1

Wild *P. vannamei* reproducers, captured at night at Jama (Manabi, Ecuador), were transported to the CENAIM research center and kept in maturation tanks for 2 to 3 weeks to acclimate to experimental conditions. After acclimation, female shrimp were unilaterally eyestalk-ablated by cutting and pinching and marking with eye-tags. A unisex system was used as described in Browdy et al. (1996): three tanks were stocked with 40 females each and three tanks with 45 males each. At the time of stocking, the average weights of the male and female shrimps were 47.5 and 63.3 g, respectively. The postablation phase of the experiment lasted 77 days, during which females with fully developed ovaries were transferred to one of the three male tanks. If females mated, they were placed in spawning tanks. If not, they were returned to their maturation tanks.

Animals were fed a base diet that consisted of fresh frozen squid, mussel, oyster, and clam at a ratio of 2.5:1.3:1:1 and at a rate of $12\% \times d^{-1}$ of the tank live biomass wet weight basis (WWB). Administering two different fresh-frozen diet supplements at a rate of $6\% \times d^{-1}$ WWB resulted in the following treatments: A and EA received a supplement of adult *Artemia* and enriched *Artemia*, respectively. Treatment SQ did not receive a separate supplement; therefore, $6\% \times d^{-1}$ WWB more frozen squid was added to the base diet. The *Artemia* were harvested from San Francisco Bay ponds by San Francisco Bay Brand Co. (CA, USA) and were enriched after harvesting according their standard procedure with an experimental emulsion provided by the Artemia Reference Center (Gent, Belgium). The booster consisted of an ICES 30/4/E reference emulsion containing 30% PUFA to which 2% cholesterol, 3,000 ppm ascorbic acid (AA) equivalent (ascorbyl palmitate; Roche, Belgium), 1,000 ppm α-tocopherol (α-TOH) equivalent (DL-α-tocopherol acetate, ATA, Roche), and 1,000 ppm astaxanthin (AX) equivalent (Carophyl Pink, Roche) were added. Feeds were administered in five daily rations, two of which consisted of the dietary supplement only. The three treatments were applied in the same way for female as for male broodstock.

The maturation tanks were oval-shaped (5 m × 3 m; 19.6 m^2) black *Fiberglas* tanks in which sand-filtered and UV-treated seawater (salinity 33 g × L^{-1}, pH 7.8–8.2) was exchanged at a rate of 250% daily. Water temperature was 24.0 °C during acclimation and was heated to 28.5–29.0 °C from ablation onward. A timer-controlled, inverted photoperiod of 14 h light:10 h dark was adopted, with gradual transition between light and dark hours. Mated females were transferred to individual 300-L black spawning tanks, and from each spawn, the eggs were hatched out in 20-L buckets. Nauplii were collected after phototaxism selection and stocked in 1-L bottles at a density of 100.L^{-1} and a temperature of 29 °C until metamorphosis to zoea 1 (Z1). Eight-day larviculture trials were run in 3-L glass bottles. Late nauplii 5 (N5) were stocked at a density of 100 ind × L^{-1} in seawater of 33 g × L^{-1} and 28.5 °C. Water was exchanged 90% daily, and an algae concentration of 100,000 cells × mL^{-1} *Chaetoceros* sp. was maintained.

The hatching percentage was estimated by concentrating the viable nauplii in a 10-L bucket and counting five subsamples. The percentage of egg fertilization was determined by the presence of a double membrane and/or embryonic development. Zoea 1 length was measured with a profile projector on samples of 30 zoea each. Spermatophore quality was based on sperm count and spermatophore weight (Alfaro and Lozano 1993).

Experiment 2

Wild reproducers were captured at San Pablo (Guayas, Ecuador) and transported to CENAIM. Acclimation and ablation techniques were similar to those used in experiment 1. Three tanks with mixed sex were monitored during 55 days postablation. Each tank was stocked with 45 males and 40 females with average weights of 55.6 and 64.5 g, respectively. A similar feeding strategy as in experiment 1 was adopted, but diet supplements were adult frozen *Artemia* enriched with three different boosters. For treatments L and LV, the oil component of the enrichment product consisted of the ICES 30/4/E reference emulsion with inclusion of 2% cholesterol. In treatment V, this oil component was replaced by the ICES 0/0/E reference emulsion based on a PUFA-free coconut oil. Furthermore, boosters V and LV contained high vitamin levels: 3,000 ppm AA equivalent, 1,000 ppm α-TOH and 1,000 ppm AX equivalent. All treatments were isocaloric.

The same infrastructure and conditions as in experiment 1 were used, but this time water temperature ranged from 27.0 to 29.5 °C. Larval monitoring was only continued up to stage Z1.

Data Processing

Female reproducers were considered as experimental units. For statistical analysis, either animals or spawns were considered as treatment replicates. Infertile spawns were not considered. In the case of ovarian maturation frequency, daily observations were used as replicates, as in Nascimento et al. (1991). Pearson moment-product correlation was used to determine correlations between the independent variables, female weight and spawn order, and the dependent variables related to spawn size and spawn quality. Data were analyzed with analysis of variance: a two-way ANOVA for experiment 1 with male tank as second variable and a one-way ANOVA for experiment 2. Female weight and spawn order were included as covariates in an analysis of covariance (ANCOVA) for the number of eggs per spawn and Z1 length, respectively, as correlations were found between them. When necessary, data expressed in percentages or fractions were asin√ transformed to obtain normal distribution, although unadjusted means are presented. Duncan's new multiple range test was used to identify differences among treatments. All references to statistical significance were at the 5% level or lower.

RESULTS

Experiment 1

Mean survival rates of 35.0, 62.5, and 82.5% were registered for male reproducers and 35, 37.5, and 47.5% for female reproducers of treatments SQ, A, and EA, respectively. On average, female reproducers survived 41 days out of the total 77 days of the experiment, for which no differences were detected among treatments. In total 9, 25, and 55 fertile spawns were obtained in treatments SQ, A, and EA, respectively.

Spawn size (eggs per spawn) was not affected by dietary treatment (Table 1). On the other hand, ovarian maturation and rematuration as well as repeat spawning differed among treatments. The maturation frequency was higher when *Artemia* (A and EA) was supplemented to the diet. The number of females with repeated spawns increased significantly in the order SQ–A–EA, spawning frequency and total egg production per female followed the same trend. Also, all parameters related to egg or larval quality were significant better in A and EA as compared to SQ (Table 2). The total nauplii production per tank after 77 days, expressed as percentages of the production in treatment SQ, were 451% and 875% for the *Artemia* and enriched *Artemia* treatments, respectively. No significant effect of the diet on spermatophore quality could be detected (sperm count, spermatophore weight presented in Table 3; egg fertilization presented in Table 1), neither was there a male tank effect in the analysis.

Experiment 2

During the 55 days postablation mean survival rates of 77% for male and 58% for female reproducers were registered. On average, female reproducers survived 45 days out of the total 55 days of the experiment, for which no differences were detected among treatments. Over the whole period, 91, 61, and 24 fertile spawns were recorded for treatments LV, L, and V, respectively.

Table 4 illustrates how different treatments affected the reproductive performance. The maturation frequency was significantly higher for spawners of treatment L than for spawners of treatment LV. No statistical testing was possible on spawn frequencies (no normal distribution), but a decreasing trend is observed in the order LV–L–V. Spawn size did not differ between treatments. Spawners that received a supplement of *Artemia* enriched with high vitamin levels only (V) exhibited the lowest number of eggs produced per female, the lowest egg fertilization, and the lowest incidence of

TABLE 1.

Effect of different dietary supplements on *P. vannamei* spawners: maturation frequency, spawn frequency, number of females that spawned more than once, fecundity, and fertilization (experiment 1).

	Dietary Supplement		
	SQ	A	EA
Maturation/female/day*	0.023 ± 0.037^{a}	0.067 ± 0.065^{b}	0.082 ± 0.068^{b}
Spawns/female/day	0.003 ± 0.009	0.011 ± 0.024	0.023 ± 0.032
# Females that spawned more then once†	7^{a}	16^{b}	36^{c}
Eggs/spawn ($\times 10^3$)	213.0 ± 67.9^{a}	214.4 ± 60.8^{a}	210.1 ± 69.1^{a}
Eggs/female ($\times 10^3$)	58.6 ± 142.9^{a}	188.9 ± 388.7^{ab}	337.3 ± 507.327^{b}
Egg fertilization (%)	47.0 ± 26.9^{a}	65.9 ± 21.6^{a}	65.9 ± 23.8^{a}

* Observation of ovarian maturation stage 3 or 4 according to King (1948).
† Statistical differences detected with χ^2 test.

repeated spawns. In Table 6, the mean sperm count in both spermatophores of male reproducers is given, a recording that was significantly higher in treatment L as compared with treatment V.

No significant effect of the dietary treatments on egg quality or larval quality was observed (Table 5). However, the number of zoea 1 produced per spawn (a combination of spawn size and larval survival) was lower in treatment V as compared with treatment PV. The decrease of larval survival with successive spawns (spawner exhaustion) seemed to be more critical in treatment V as in the remaining treatments (Fig. 1).

DISCUSSION

A dietary regime consisting of squid, oyster, clam, and mussel (treatment SQ) gave poor results for most reproductive parameters. This is most probably attributed to the noninclusion of bloodworm (Middleditch et al. 1980, Lytle et al. 1990). Supplementation with *Artemia* biomass resulted in higher survival, improved maturation and reproduction, and better offspring quality. Lavens and Sorgeloos (1991), Cahu et al. (1991), and Palacios et al. (1998) demonstrated with their work on *Macrobrachium rosenbergii*, *P. indicus*, and *P. vannamei*, respectively, that offspring quality is associated with the level of metabolic fuel (mainly lipids) in eggs and nauplii. Because fuel levels in eggs or nauplii depend on the nutritional status of the female broodstock, which is affected by their dietary regime, we can attribute the positive effect of *Artemia* biomass on egg and larval quality to its good nutritional value. The nutritional composition of adult *Artemia* is well documented in reviews by Léger et al. (1986) and by Lavens and Sorgeloos (1996). It seems to be quite similar to the body composition of penaeid shrimp, and therefore, it is likely to contain appropriate protein and lipid levels. Considering that biomass of adult *Artemia* was used in this study, its effect on ovarian maturation and reproductive activity might also be attributed to hormones or sexual steroids in addition to the nutritional input. Indeed, it is likely that the reproductive hormones within crustaceans are of the same nature and, therefore, could be effective in other species. The findings of Mendoza et al. (1997) on the effect of squid extracts on vitellogenesis in *P. vannamei*, and of Alava and Kanazawa (1991) on the effect of clam on ovarian maturation in *P. japonicus*, suggest a role of methanol–water-soluble extracts (hormones, steroids) on shrimp maturation. Further research on this topic could help with the identification of the *Artemia* component responsible for triggering maturation.

Supplementing the feeding regime with enriched *Artemia* as compared to regular *Artemia* promoted mating and spawning. Also, a positive effect on maturation seems to exist, but because of high within-treatment variation, no significant differences were detected. The booster emulsion was particularly rich in lipids, which provide essential nutrients as well as energy. Our studies on the biochemical composition of wild *P. vannamei* reproducers (Wouters et al. in preparation) as well as studies on other shrimp species (Middleditch et al. 1979; Read and Caulton 1980; Jeckel et al. 1989, Castille and Lawrence 1989, Mourente and Rodriguez 1991) demonstrated a remarkable increase of lipids in the ovaries

TABLE 2.

Effect of different dietary supplements on mean percentage hatch, percent larval survival from nauplii 2 to zoea 1, number of zoea 1 per spawn, zoea 1 length and percentage larval survival from zoea 1 to mysis 2 (experiment 1).

	Dietary Supplements		
	SQ	A	EA
Hatch (%)	31.6 ± 36.4^{a}	60.8 ± 27.5^{b}	61.6 ± 30.2^{b}
Larval survival N2-Z1 (%)	41.1 ± 32.0^{a}	71.8 ± 22.2^{b}	69.4 ± 21.1^{b}
Zoea 1/spawn ($\times 10^3$)*	42.8 ± 56.5^{a}	93.4 ± 60.7^{b}	94.0 ± 60.8^{ab}
Zoea 1 length (μm)	804.7 ± 116.3^{a}	900.5 ± 36.5^{b}	884.4 ± 36.4^{b}
Larval survival Z1-M2 (%)	8.3 ± 4.7^{a}	43.9 ± 23.7^{b}	48.4 ± 19.5^{b}

* Calculated data (number of nauplii per spawn × larval survival/100).

TABLE 3.

Spermatophore quality of *P. vannamei* male reproducers at the end of experiment 1 estimated by mean sperm count (number of sperm cells in both spermatophores) and mean spermatophore weight.

	Dietary Supplements		
	SQ	A	EA
Sperm count ($\times 10^6$)	12.97 ± 12.89^{a}	13.44 ± 11.59^{a}	13.56 ± 6.75^{a}
Spermatophore weight (g)	0.0397 ± 0.0161^{a}	0.0827 ± 0.0468^{a}	0.0682 ± 0.0208^{a}

TABLE 4.

Effect of different enriched *Artemia* supplements on maturation frequency, spawn frequency, number of females that spawned more than once, fecundity, and fertilization of *P. vannamei* spawners (experiment 2).

	Artermia Supplements		
	LV	L	V
Maturation/ female/day*	0.141 ± 0.066[a]	0.173 ± 0.079[b]	0.140 ± 0.080[ab]
Spawns/female/day	0.064 ± 0.052	0.048 ± 0.052	0.025 ± 0.035
# Females that spawned more then once†	28[a]	19[a]	11[b]
Eggs/spawn (× 10^3)	288.1 ± 98.4[a]	296.6 ± 100.1[a]	278.5 ± 69.0[a]
Eggs/female (× 10^3)	865.8 ± 577.3[a]	717.6 ± 467.4[a]	371.4 ± 142.9[b]
Egg fertilization (%)	71 ± 27[a]	64 ± 24[ab]	41 ± 16[b]

* This is the maturation frequency: observation of ovarian maturation stage 3 or 4 according to King (1948).
† Also referred to as rematuration; Statistical differences detected with χ^2 test.

during maturation. In addition, maturation and reproduction cause increased metabolic energy demands (Harrison 1990), which can be met by augmenting maternal nutrition (Clarke 1982, Bray et al. 1990). The results of the present study indicate that through the enrichment of *Artemia* more adequate lipid levels were obtained that could sustain optimal reproductive performance of *P. vannamei*.

Experiment 2 further confirms that the role of the enrichment product is not only energetic but also nutritional, all treatments were iso-caloric. Most studies on shrimp broodstock nutrition have focused on the use of various natural diets, but few attempted to elucidate specific nutritional requirements (Harrison 1990) and/or possible effects on maturation, spawning, or offspring quality. Studies on lipid requirements indicate the importance of polyunsaturated fatty acids (PUFA; Middleditch et al. 1980, Chamberlain 1988, Jeckel et al. 1989; Lytle et al. 1990; Teshima and Kanazawa 1983, Xu et al. 1994; Cahu et al. 1995) and cholesterol (Middleditch et al. 1980; Kanazawa et al. 1988). Others studied the role of vitamins in shrimp reproduction: ascorbic acid (Cahu et al. 1991, Alava et al. 1993a, Cahu et al. 1995), alpha-tocopherol (Chamberlain 1988, Alava et al. 1993b, Cahu et al. 1995), and

TABLE 5.

Mean percentage hatch, percent larval survival from nauplii 2 to zoea 1 (N2-Z1), number of zoea 1 per spawn and zoea 1 length for the three *Artemia* treatments (experiment 2).

	Artemia Supplements		
	LV	L	V
Hatch (%)	50.9 ± 33.6[a]	44.4 ± 29.4[a]	32.2 ± 24.6[a]
Larval survival N2-Z1 (%)	62 ± 24[a]	54 ± 30[a]	47 ± 27[a]
Zoea 1/spawn (× 10^3)*	87.2 ± 84.4[a]	69.2 ± 75.5[ab]	40.6 ± 42.2[b]
Zoea 1 length (μm)	895.7 ± 116.3[a]	862.6 ± 193.5[a]	799.9 ± 264.9[a]

* Calculated data (number of nauplii per spawn × larval survival/100).

TABLE 6.

Spermatophore quality of *P. vannamei* male reproducers at the end of experiment 2 estimated by mean sperm count (number of sperm cells in both spermatophores) and mean spermatophore weight.

	Artemia Supplements		
	LV	L	V
Sperm count (10^6)	11.05 ± 4.24[ab]	28.72 ± 2.40[a]	12.37 ± 4.50[b]
Spermatophore weight (g)	0.0956 ± 0.0172[a]	0.0879 ± 0.0422[a]	0.0836 ± 0.0175[a]

vitamin A (Alava et al. 1993b, Dall 1995). Also, astaxanthin can be considered as a vitamin (it may serve as a vitamin A precursor), and several biochemical studies have detected considerable levels of this carotenoid in hepatopancreas and gonads of crustacean reproducers (Vincent et al. 1988, Dall 1995, Dall et al. 1995; Sagi et al. 1995, Mantiri et al. 1996). The second experiment does not allow determination of the optimum dietary levels of each enrichment component, but may give us an estimation of the importance and role of the vitamin and lipid fractions in penaeid reproduction. Best results were obtained with the treatment that received a supplement of *Artemia* biomass enriched with lipids and vitamins (LV). However, by reducing the concentration of PUFA and cholesterol in the enriched *Artemia*, a decline in egg fertilization, a lower incidence of repeated spawns, and a lower egg production per female was observed, which clearly demonstrates the importance of PUFA and cholesterol.

Obviously, good reproductive performance also depends on male testis maturation and spermatophore quality. Several authors evaluated spermatophore quality by monitoring such parameters as spermatophore regeneration, spermatophore weight and color, sperm count, and percentage of abnormal and dead sperm cells (Chamberlain 1988, Leung-Trujillo and Lawrence 1991, Alfaro and Lozano 1993). Chamberlain detected a dietary effect on spermatophore quality. Others adopted a unisex system to detect male diet effects on mating and fertilization but failed to do so (Naessens et al. 1997). In experiment 1 of the present study, no male effect was observed (Table 3), but in experiment 2, significant differences in mean sperm count were detected at the end of the experiment (Table 6). The sperm count in treatment L was twice that of the remaining treatments, but only significantly different from treatment V. The latter result suggests a negative effect of high vitamin levels on sperm production. The nonsignificant differences with the LV treatment might be explained by the fact that part of the vitamins were used as antioxidative agents protecting the high PUFA levels. In contrast, Chamberlain (1988) reported a positive effect of vitamin E on spermatophore quality; he detected lower percentages of abnormal and lysed sperm cells in a treatment that received a diet high in vitamin E (approximately 500 mg/kg ATA) and suggested that this is related to the membrane stabilizing properties of tocopherol. It is possible that the vitamin concentrations in the *Artemia* biomass of treatments LV and V were excessively high, causing oversaturation or hypervitaminosis, particularly of fat-soluble vitamins. This would also explain the observed treatment differences in frequencies of ovarian maturation of the female reproducers. However, vitamins and astaxanthin do seem to play a positive role on shrimp reproductive performance if they are provided together with high PUFA and cholesterol levels:

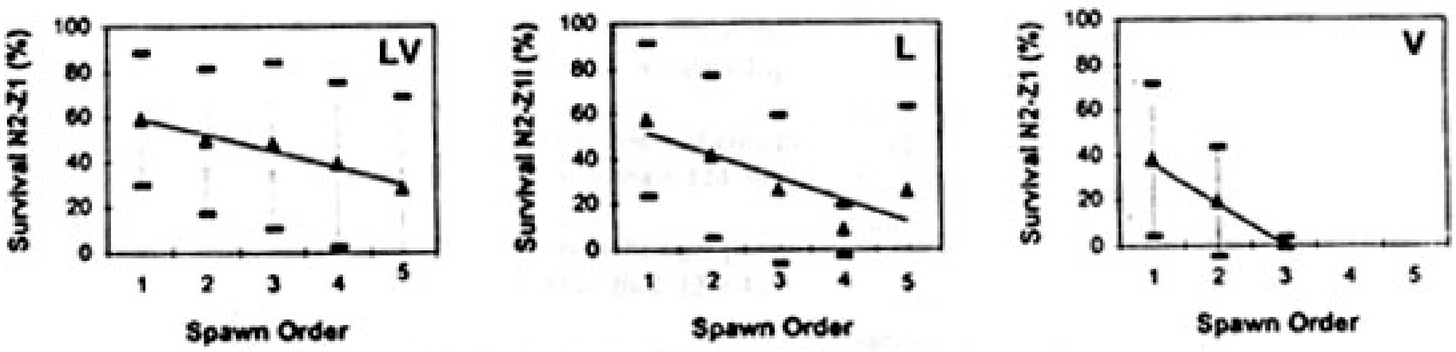

Figure. 1. Mean percentage larval survival from Nauplii 2 to Zoea 1 according spawn order for the three dietary treatments of experiment 2. Best fitted lines and standard deviation bars are shown.

the best performing treatment in most aspects is the one that received the LV enriched *Artemia*. We assume that the positive role of high vitamin levels can be attributed to their antioxidant properties. Cowey et al. (1985) demonstrated that vitamins C and E are very efficient lipid antioxidants in eggs of salmon. Also Cahu et al. (1995) suggest that the beneficial action of vitamins C and E can be found in their antioxidant properties, through the protection of biological membranes from oxidation and degradation by free radicals. Further research on the role of vitamins and astaxanthin and their optimal diet inclusion levels would help to formulate performing broodstock diets.

It is interesting to note that between treatment EA of experiment 1 and treatment LV of experiment 2, which were fed similar diets, very distinct values were recorded for percentage hatch, larval survival, and zoea length. The wild reproducers used in this study were obtained at different times of the year (June to July 1996 and February to March 1997) and at different locations (Jama and San Pablo). Hansford and Marsden (1995) and Marsden et al. (1997) also reported high variability in reproductive performance between *P. monodon* prawns captured at different seasons and explained this by differences in age and environmental effects. In the present study, spawners of both experiments had similar weights and, therefore, presumably similar ages. As such, the observed differences are probably attributable to seasonal–environmental and/or geographical effects. For reproducers of experiment 1, a high mortality was recorded during the purchase as well as a high occurrence of necrosis. It seems that the adopted acclimation period (2–3 weeks) on natural diets did not help to overcome the initial poor condition and poor nutritional status of the spawners used in experiment 1.

Finally, it is observed that spawner exhaustion; that is, decreasing larval survival with successive spawns, was less pronounced in treatments LV and L as in treatment V. In the latter treatment, no larval survival was obtained from spawns of the third order. This is a clear indication that rematuration and repeat performance can be promoted by providing adequate diets, a finding that is in agreement with the reported data for *P. monodon* by Marsden et al. (1997).

CONCLUSION

Supplementing regular *Artemia* biomass to *P. vannamei* broodstock diets promotes maturation and spawning, and improves larval quality. Better results are obtained if animals are co-fed with *Artemia* biomass that is enriched with high levels of polyunsaturated fatty acids (PUFA) and cholesterol, vitamin C, vitamin E, and astaxanthin. The PUFA-cholesterol fraction of the booster emulsion plays the most important role, improving egg fertilization and promoting spawning. A positive role of the vitamin fraction can be obtained if combined with high levels of oxidative products (PUFA and cholesterol), but too high a vitamin level may cause oversaturation and, thus, negative effects. Therefore, including enriched *Artemia* biomass daily in the broodstock feeding regime at a rate of 6% of the tank biomass (WWB) or higher can be recommended.

ACKNOWLEDGMENTS

This study was supported by the VLIR-Eigen Initiatieven Program of the Flemish Inter-University Council. The authors thank San Francisco Bay Brand Co. and INVE, Inc. for providing the *Artemia* products and Marcelo Hidalgo (CENAIM) and Karla Van Ryckeghem (ARC) for their technical assistance. We also thank the following students for their interest and participation in the experiments: Juan Valdivieso, Sylvia Alvarez, Alberto Torres, Juan Carlos Vasquez, and José Vergara.

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